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Antimicrobial Study of Whole Extract, Isolated Ingredient, and Newly Synthesized Analogues from *Cassine glauca* Plant

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ABSTRACT

Life co-existed with disease, decay and death. The study of disease and their treatment is related with the human intellect. Illness has been the man's heritage from the beginning of his existence and the search for remedies to combat the diseases is perhaps equally old. Melghat region is a rich source of medicinal flora. *Cassineglauca* is one of the medicinally potential plants found in Melghat region which is frequently used for the eradication of skin diseases[1]. The present investigation was undertaken to screen the efficacy of *Cassineglauca* whole extract, isolated acidic ingredient and its newly synthesized analogues on certain bacteria which are responsible for skin diseases such as *Streptococcus pyogenes*, *Nocardia calcarea*, *Bacillus subtilis*, and *Pseudo monasaeruginosa*. In comparison among the impact of test compounds; it was revealed that, the *Cassine glauca* isolated acidic ingredient and its newly synthesized analogues showed significant activity than the whole extract.

Keywords: *Cassineglauca*, Melghat, Antimicrobial Study

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INTRODUCTION

In order to explore the medicinal potential of plant kingdom we have chosen Melghat forest area which is one of the richest biological heritage lying at the northern extreme of the Amravati district of Maharashtra situated on the border of Madhya Pradesh, in the south western Satpura Mountain ranges. The flora of Melghat has tremendous medicinal potential to cure hundreds of human ailments[2]. This very fact prompted and encouraged us to undertake the present study of exploration of efficacy of medicinal flora of Melghat forest region with special reference to their remedial impact on skin diseases. This is so because now a days skin disease is a world wide problem especially in developing and underdeveloped countries. Generally Eczema, Scabies, Ringworm, Clavus or Corn, Prurigo, Yaws, Warts, Pruritis and Psoriasis are common skin diseases found in tropical and subtropical countries[3]. In the literature, the *Cassine glauca* tree though not abundant anywhere is fairly scattered throughout India and is commonly found in mixed deciduous forests. The tree prefers clay soils. It is a moderate shade bearer and is fairly frost-hardy. Various parts of this tree are used in the indigenous system of medicine. The root is used as an antidote to snake bite. It is emetic if taken internally; it is fatal in over doses. The ground root made into a paste is applied to swellings. The powdered leaves have a sternutatory action; they relieve headache. They are also useful in treating hysteria. A decoction of leaves is given for eczema and other skin affections. The leaves contain β-sitosterol and lupeol[4].

MATERIALS AND METHOD

The leaves of *C. glauca* plant was collected from the Melghat region of Amravati, District of Maharashtra, India seasonally and authenticated by the taxonomists Dr. S. P. Rothe from the Department of Botany, Shri Shivaji College Akola. Voucher specimen (ML– 104) was deposited in the herbarium of the College.

Chemicals

All the chemicals used in the study were obtained commercially and of analytical grade.

Microorganisms

The test organisms *Streptococcus pyogenes, Nocardia calcarea, Bacillus subtilis,* and *Pseudomonas aeruginosa* were procured from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune 411 008.

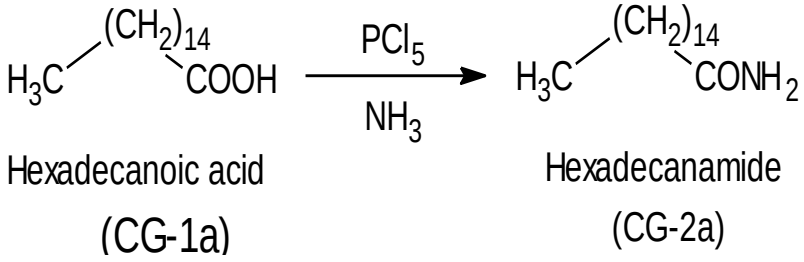
EXPERIMENTAL

Soxhlet extraction method is used for the preparation of extracts of *Cassine glauca* plant material. The coarse powders of leaves, stem, flowers, seeds and roots of *Cassine glauca,* were extracted

with water and acetone solvents by using soxhlet apparatus. These extracts were concentrated at 40 ^{o}C using rotary evaporator. The extracts thus obtained were dried and stored separately in air tight bottles for further study. The acidic ingredients present in the extracts were separated by conventional as well as sophisticated chromatographic methods. The major active acidic ingredient i.e. Palmitic acid (CG-1a) separated from acidic ingredients was then used for the preparation of analogues[5-7].

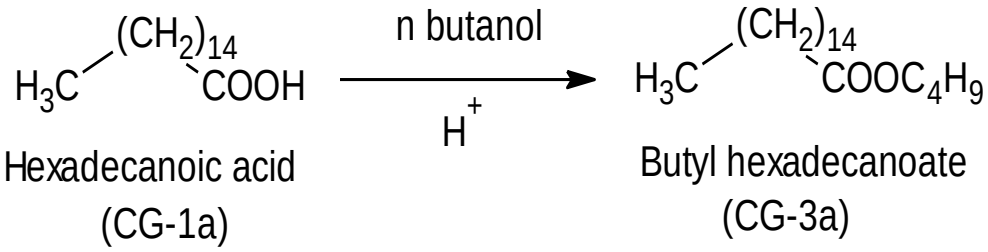
Preparation of hexadecanamide (CG-2a) from hexadecanoic acid (CG-1a)

Hexadecanoic acid (Palmitic acid) (CG-1a) was mixed with PCl_5 (1:4) solid in a dry porcelain dish. The mixture was grinded with the help of apestle, in the fume cupboard, till it liquefied. The liquid, so obtained, was the crude acid chloride. The acid chloride was then treated with Conc. NH_4OH. A vigorous reaction took place. The product was cooled, filtered and washed with water. The product was then recrystallized with alcohol to get hexa decanamide (CG-2a). M. P. 106 ^{o}C, Yield: 75 %.



Preparation of butyl hexadecanoate (CG-3a) fromhexadecanoic acid (CG-1a)

Hexadecanoic acid (Palmitic acid) (CG-1a) was refluxed with n-butanol (1:2) in presence of H_2SO_4 for 6-8 hours. The mixture, thus obtained, waswashed with water and distilled to get the product hexadecanoic butylester (CG-3a). B. P. 102 ^{o}C, Yield: 80 %[8-11].



The isolated active ingredient and synthesized analogues were characterized on the basis of elemental analysis, molecular weight determination, and spectral data[12-15]: The details thereof areas ***COMPOUND (1A): Yield:*** 45%, ***M.P.*:** 65 °C, ***Elemental analysis for*** **C16H32O2:** Found C =74.94, H = 12.58, O = 12.48, Calculated C = 74.94, H = 12.60, O = 12.49 %. ***UV spectrum (EtOH):***λmax227 nm (n→Π*), 218 nm (Π→Π*).***IR spectrum, ν, cm–1:*** 3091-2532(-COOH stretching), 2956(aliphatic –C-H stretching), 1701 (-C=O stretching), 1294 (-C-O stretching). ***1H***

NMR spectrum (400 MHz, CDCl3): δ ppm 0.90 (3H, t, C**H3**-CH_2-), 1.29 (24H,bs, (–C**H2**-)**12**-CH2), 1.64(2H, m, –C**H2**-CH2), 2.36 (2H, t, –CH2-C**H2**-COOH).

COMPOUND (2A):Yield: 75%, ***M.P.:*** 106 °C, ***Elemental analysis for*** $C_{16}H_{33}NO$**:** Found C = 75.23, H = 13.02, O =6.26, N = 5.48, Calculated C = 75.25, H = 13.04, O = 6.27,N = 5.49%.***UV spectrum (EtOH):*** λmax214 nm (π→π*);***IR spectrum v, cm–1:*** 3387 (-NH2 stretching), 2951-2846 (-C-H stretching), 1643 (-C=O stretching in amide), 1188,1120 (C-O stretching). ***1H NMR spectrum (400 MHz, CDCl3):*** δ ppm 0.88 (3H, t, C**H3**-CH2-), 1.25(22H, s,An envelope of 22 hydrogens CH_3-(C$\mathbf{H_2}$)$_{11}$-), 1.61 (2H, m,–C$\mathbf{H_2}$$CH_2CH_2CONH_2$),2.21 (2H, t, –$CH_2C\mathbf{H_2}$$CH_2CONH_2$), 2.31 (2H, t,–$CH_2CH_2C\mathbf{H_2}$$CONH_2$), 5.47(1H, s, -N**H)**, 5.94(1H, s, -N**H)**.

COMPOUND (3A):**Yield:** 80%, **M.P.:** 102 °C, **Elemental analysis for** $C_{20}H_{40}O_2$**:** Found C =76.86, H = 12.90, O = 10.24, Calculated C = 76.88, H = 12.91, O =10.25 %.**UV spectrum (EtOH):**λmax255 nm (n→π*), 223 nm (π→π*). **IR spectrum, v, cm–1:** 3459(Overtone of >C=O), 2957, 2924, 2854(C-H stretching),1739 (>C=O stretching), 1173 (-C-O stretching).**1HNMR spectrum (400 MHz, $CDCl_3$):**δ ppm 0.88 (6H, t, C**H3**-CH_2-), 1.32 (2H, m, CH_3C$\mathbf{H_2}$$CH_2CH_2OCO$-),1.45 (2H, m, -$CH_2C\mathbf{H_2}$COO-),3.34 (2H, t, CH_3CH_2C$\mathbf{H_2}$$CH_2OCO$-), 3.43 (2H, t, $CH_3CH_2CH_2$C$\mathbf{H_2}$OCO-), 3.86 (26H, s, An envelope of 26 hydrogens CH_3-(C$\mathbf{H_2}$)$_{13}$-).

Table 1: Antibacterial activity of test compounds

Extracts & analogues	Concentration	Inhibitory zones in mm			
		S. pyogenes	***N. calcarea***	***T. cutaneum***	***R. rubra***
1) Crude acidic ingredients	0.01 mol	12	14	12	10
2) Active acidic ingredient (1a)	0.01 mol	18	17	16	20
3) Analogue (2a)	0.01 mol	22	26	23	25
4) Analogue (3a)	0.01 mol	19	21	20	19
5) Whole extract	0.01 mol	12	14	12	10
6) *Ampicilin*disc	0.01 mol	16	20	17	16
7) *Vancomycin*disc	0.01 mol	16	20	17	18

Antimicrobial Assay[16]

The crude acidic ingredients, major acidic ingredient (1a), analogues prepared (2a & 3a) along with whole extract of leaves were screened for their antibacterial potency by cup plate agar method4,5 against bacterial species *viz., Streptococcus pyogenes, Nocardia calcarea, Trichosporoncutaneum,* and *Rhodotorularubra*. The petriplates were prepared with 25ml sterile Mueller Hinton Agar. A sterile cork borer (8 mm) was used to make wells in each plate.1 ml inoculums' suspension was swabbed uniformly over the agar medium to get uniform distribution of bacteria. After labeling the plates 100µl of each test compound (at concentration of 0.01 mol)

was added aseptically into the wells. The petriplates were then incubated at 37°C for 24 hrs during which the activity was evidenced by the presence of zone of inhibition surrounding the well. The negative control was prepared using respective solvent. *Ampicilin disc* (10 mcg/disc) and *Vancomycin disc* (30 mcg/disc) were used as positive control. The zones of inhibition were recorded in millimetres by using Himedia Zone Reader Scale.

RESULTS AND DISCUSSION

The results obtained for the antibacterial test performed with analogues prepared from isolated acidic ingredient along with mixture of acidic ingredients and whole extract of *Cassine glauca* plants presented in Table 1. The analogues prepared were found to be most effective against all pathogenic bacteria. The isolated active acidic ingredient was found to be more effective as compared with the mixture of crude acidic ingredients. The whole extract of plants found to be moderately active. The results obtained were assessed on their comparison with the activities of standard antibacterial agent like *Ampicilin* and *Vancomycin* as control.

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